THE EFFECTS OF DOSE AND TIME OF ADMINISTRATION OF PENTYLENETETRAZOL ON A VISUAL DISCRIMINATION TASK

An abstract of a thesis by
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The problem: The purpose of this study was to determine if a given range of pentylenetetrazol doses differentially affected retention of a visual discrimination task in the rat as a function of time of administration after training was completed.

Procedure: Using food reinforcement, 36 rats were trained to perform a brightness discrimination in a Y-maze until they reached a criterion of nine out of ten correct responses. They were then injected with either 10.0, 12.5 or 15.0 mg/kg pentylenetetrazol at intervals of either 0, 5 or 15 minutes after completion of training. Twenty-four hours later a test of retention was given. This consisted of counting the number of errors made by each rat in 15 additional trials run under identical conditions in the Y-maze as on the previous day. Using the number of errors made by each rat as the dependent variable, the data was analyzed by a two-factor completely randomized analysis of variance.

Findings: The results of this study indicated that the number of errors made by the rats was dependent upon the dosage of drug and the time intervals after training at which it was administered. Overall, pentylenetetrazol was shown to facilitate retention of the brightness discrimination task. In addition, it was found that the effectiveness of the drug in facilitating retention decreased linearly with increasing delay of injection.

Conclusion: It was concluded that although pentylenetetrazol in the range of doses investigated facilitates retention for a learned task, there exists a critical period after training has been completed after which injections of the drug lose their utility in facilitating retention. Within this critical period, there is a linear decrement in the drug's effectiveness with increasing delay of injection after completion of training.

Recommendations: It is recommended that procedural guidelines and a system for the standardization of variables be formulated and adhered to when conducting experiments of this sort. This would most likely serve to eliminate various discrepancies, both major and minor, now found in the results of these experiments.

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CHAPTER I

INTRODUCTION AND REVIEW OF THE LITERATURE

The influence of a drug on behavior was first quantifiably described by Karl Lashley, who found that low doses of strychnine sulfate would facilitate the learning of a maze by rats who were injected prior to the learning situation (Lashley, 1917). Thus the foundation for various hypotheses that drugs might influence learning and/or be useful in exploring memory processes was established. The implications of Lashley's experiment and a conformation of such was largely ignored for several decades. It was not until 1959, that Lashley's findings were confirmed by several studies, most notably by McGaugh, who showed that strychnine sulfate, injected shortly before daily training trials in low doses could facilitate learning in rats (McGaugh & Petrinovich, 1959).

Since 1959, there has been a great deal of research performed which has attempted to modify memory processes by administering strychnine, as well as numerous other drugs such as picrotoxin, puromycin, pentylenetetrazol and magnesium pemoline which affect central nervous system functioning. This approach is based on the assumption that an understanding of the nature of the facilitating, or with certain drugs the inhibitory, effect of the treatment on memory considered together with knowledge of the mechanisms

of action of the treatment might provide important clues to the neurobiological processes underlying memory (McGaugh & Dawson, 1971).

It must be reemphasized that in the Lashley (1917) and McGaugh and Petrinovich (1959) studies, animals were given strychnine before their first encounter with the training and were trained under the influence of the drug.

McGaugh and Petrinovich (1965) point out that the most crucial problem in research concerning drug affects on learning and memory is that of distinguishing drug effects on learning from other effects of drugs on performance. Since drugs injected shortly before training could be acting on learning and/or memory processes as well as on performance variables such as motivation, arousal and/or sensory processes, it is difficult to draw conclusions regarding the effects of drugs on learning and memory when the drugs are injected shortly before training.

It is for this reason that, generally, Ss in such experiments are first trained on a task and are then treated either immediately or at some time later. Then, at some time after the animals have recovered from the acute effects of the treatment, retention tests are given. Inferences concerning the effects of the treatment on memory storage processes are based on retention performance. Under ideal circumstances the animals are neither trained nor tested while under the acute effects of the drug.

In line with the above criteria, McGaugh (study reviewed by Breen & McGaugh, 1961) was able to show that strychnine when administered shortly after training trials also facilitated learning, and hence memory.

McGaugh's findings were important in that they provided the beginning of the attempt to correlate the possible relationships between drugs, memory, and learning. Since then many different studies have been done attempting to facilitate or inhibit learning by treating animals with various drugs. As might be expected the degree of facilitation or inhibition obtained depends upon many factors including not only the type of animal used, but the strain of the particular animal, the particular drug used, the dosage of that particular drug, the type of training task, the complexity of the training task, and the interval between termination of training and drug administration or injection (Krivanek & McGaugh, 1968; McGaugh & Dawson, 1971). In some instances the relationship between learning as either facilitated or inhibited via "strengthening or weakening" memory processes has been shown to involve an interaction of two or more of the above variables (Hunt & Bauer, 1969; McGaugh & Dawson, 1971).

After finding facilitation of learning with posttrial injections of strychnine sulfate (reported in Breen & McGaugh, 1961), McGaugh set out to extend his findings to other drugs. It was found that the drug picrotoxin could

facilitate maze-learning in rats when they were injected with 1.00 to 1.25 mg/kg of picrotoxin 30 seconds after each trial. The rats used in this study were of two types, a "maze-bright" strain and a "maze-dull" strain. Maze-dull Ss, injected after each trial, made significantly fewer errors than saline-injected maze-dull controls. Maze-bright Ss did not differ from the saline-injected maze-bright controls. In addition, no significant improvement in maze learning was found for doses under 1.00 mg/kg (Breen & McGaugh, 1961).

Petrinovich, Bradford, and McGaugh (1965), using two different strains of rats, found that strychnine when administered each day after either the first or second of four trials on a delayed-alternation problem with long intertrial delays, facilitated learning on the trials following the administration of strychnine for both strains of rats.

Posttrial administration of strychnine and picrotoxin has been shown to enhance the ability of mice to learn a shuttlebox avoidance task, but only if the treatment was given in close temporal contiguity with the training session (Bovet, McGaugh, & Oliverio, 1966).

Conflicting results for the facilitation of a one-way avoidance response have been found for the drug magnesium pemoline. Cyert, Moyer, and Chapman (1967) found no significant increase in the learning of a one-way

avoidance response, while Thompson and Knudson (1968) found that magnesium pemoline facilitated learning of this response. Discrepancies here may have resulted from different doses used in the two studies.

In addition to the facilitating effects of strychnine, picrotoxin and magnesium pemoline on memory storage, similar effects have been found for certain other drugs such as caffeine, amphetamine, nicotine, diazadamantanol, physostigmine and pentylenetetrazol (McGaugh & Dawson, 1971).

Several drugs used in memory-learning research, such as cycloheximide, (Geller, Robustelli, & Jarvik, 1971), acetoxycycloheximide, (Cohen & Barondes, 1968), and most notably puromycin (Flexner, Flexner, & Stellar, 1963; Agranoff & Klinger, 1964; Barondes & Cohen, 1966; Davis, 1968; and Flexner & Flexner, 1968) have been shown to be memory-inhibitors for a variety of behavioral tasks such as avoidance learning and discrimination learning. As with the memory-enhancers, the inhibitory power of these drugs has been shown to be a function of dose, task, organism studied, etc.

Flexner and Flexner (1968) showed that after mice had learned a maze, injection of puromycin one or more days later disrupted that learning. In addition, they found that when puromycin was administered either before or immediately after training, the ability of the mice to learn the maze was unimpaired, but the ability to retain what they had

learned was impaired. Thus, it was concluded that puromycin interfered with memory consolidation.

When puromycin was injected intracranially into goldfish, it was found to produce impairment of memory for the shock-avoidance task response of swimming over a barrier to the "safe-side" compartment of a tray when a light cue was presented. All fish were trained in this task and immediately after training, half of the fish were injected with puromycin. Seventy-two hours later, all fish were again tested. Those injected with puromycin showed no significant improvement and made significantly fewer correct responses than the control group. Consistent with Flexner and Flexner (1968), it was found that when puromycin was injected 72 hours before training, there was no effect on training and/or learning. In addition, it was decided to establish whether puromycin exerted an effect on the performance of trained or "over-learned" goldfish. When the over-learned fish were injected with puromycin and tested 72 hours later, the results indicated that puromycin had no effect on their performance or memory (Agranoff & Klinger, 1964).

Agranoff and Klinger's study is interesting in that it lends support to the "dual-trace" memory hypothesis (Hebb, 1949). Essentially, what this hypothesis assumes is that memory storage processes are "time-dependent", and that short-term memory and long-term memory are based on

different processes. According to Hebb, the input of stimulation produces "reverberating" neural activity representative of a particular learning experience, which persists for a short (though unspecified) period of time and corresponds to what is known as short-term memory. While this reverberatory activity lasts, the permanent structural change underlying the long-term memory is slowly developing. Once the reverberatory trace dies out, the structural change stops but remains at the level attained having been consolidated into long-term memory. Implicit in this theory is that drugs can in some way modify neural activity either by facilitating or inhibiting memory consolidation.

Thus, puromycin can be thought to be somehow a depressor of the reverberating neural activity or short-term memory necessary to achieve memory consolidation or long-term memory as Agranoff and Klinger showed by injecting their fish immediately after training and thus causing a depression of memory consolidation. The fact that puromycin did not affect the over-learned fish can be explained by assuming that memory consolidation for the avoidance task was already complete at the time of injection and not susceptible to disruption. This is consonant with the hypothesis that the time of injection after the initial training session was beyond some critical period of time or labile period in which the memory trace could be affected. The fact that the puromycin had been rendered metabolically inactive

when the organism reached the labile period.

In an attempt to account for the facilitating mechanisms of certain drugs, McGaugh (1966) has proposed that a stimulating experience is transformed into a memory trace which goes through an intermediate storage stage from which information is either lost or consolidated into permanent memory. The simplest explanation of drug facilitation effects is that drugs either speed up the consolidation process or retard the rate at which information drops from the intermediate memory stage prior to consolidation. Thus, one should expect maximum facilitation from the drug if it were to be introduced immediately following training and would expect the drug to have progressively less effect if its introduction were delayed.

Thus, it is assumed that certain drugs when given in certain doses after training will facilitate performance on subsequent tests of retention, and that these drugs act by facilitating memory consolidation (McGaugh & Dawson, 1971). Implicit in the above is that memory fixation is an active process extending for some time after training.

Since 1959, several investigators found that learning could be enhanced by low doses of several convulsive drugs. Some research using two of these drugs, strychnine and picrotoxin, has already been presented. A third drug, pentylenetetrazol or Metrazol based upon a report of the research that follows, appears to be one of the most

effective drugs for the enhancement of learning in rats and mice investigated thus far (Krivanek & McGaugh, 1968).

Using mice, Irwin and Benuazizi (1966) compared the ability of strychnine, picrotoxin, and pentylenetetrazol to enhance learning and/or memory retention in a one-trial avoidance learning situation. Mice were placed in a box that was divided into two compartments. The two compartments were separated from one another by a hurdle. Upon crossing the hurdle, the mice received a foot shock (0.2 ma, 2 sec.) and were immediately administered either picrotoxin, strychnine or pentylenetetrazol at various doses. All animals were tested for retention (second trial) 27 hours later. It was found that memory retention, as measured by the latency of the barrier crossing response, was improved by all three drugs as compared to a previously-run group of mice that had received no drug treatment. However, the most pronounced improvement in retention for the avoidance task was by those animals that had received pentylenetetrazol.

Likewise, Krivanek and Hunt (1967) showed that rats given posttrial injections of pentylenetetrazol and strychnine showed improved learning of a brightness discrimination in a simple Y-maze. The effect of pentylenetetrazol was the greater of the two.

Krivanek and McGaugh (1968) report a series of experiments designed to test the effects of pentylenetetrazol on memory storage in mice. They are as follows:

(1) Male Balb/c and male C57B1/6 mice were used to study the effects of posttrial injection of pentylenetetrazol on learning in a Lashley III maze. The mice from each strain were divided into four groups and were given one trial per day for seven consecutive days in the Lashley III maze. Immediately after each daily trial, the mice received an intraperitoneal (i.p.) injection of saline or a solution of pentylenetetrazol in saline. Three doses of pentylenetetrazol were used: 5, 10, and 20 mg/kg. Facilitation of learning was found with both the 5 and 10 mg/kg doses of pentylenetetrazol. However, the degree of facilitation found at these two doses depended upon the strain of mice.

(2) In a study using aversive motivation, mice were placed in a Y-maze that had been partially filled with water. The mice readily learned to escape by swimming to the end of either arm of the maze whereby they climbed a small ladder and escaped onto a platform. Two light sources were placed at the end of each arm and prior to each trial, the light at the end of one alley was turned on. After determining an animal's preference for escape (light-on or light-off arm), each mouse was trained to swim to its non-preferred brightness in order to escape. During training, all animals were given six massed trials a day, until each reached a criterion of six consecutive correct choices. After reaching criterion, each animal was reversed so that

it could now escape only by swimming to the alley which was not rewarded during the original training. Reversal training was terminated when the animals reached a criterion of six consecutive correct choices. Throughout the experiment, all animals received an i.p. injection of either saline or one of two pentylenetetrazol solutions immediately after the last daily trial. The two doses used were 10 and 20 mg/kg. The results of the study showed that although the effect of pentylenetetrazol on the initial brightness discrimination was not large, the 10 mg/kg group did better than the saline controls but the 20 mg/kg group did not. However, there was a clear facilitation of the discrimination reversal task. In this part of the experiment, both the 10 and 20 mg/kg groups did markedly better than the saline controls, apparently suggesting that the degree of facilitation varied with the task difficulty as well as the dose.

(3) Using an appetitively motivated visual discrimination task in a Y-maze, an attempt was made to explore the range of effective pentylenetetrazol doses for Swiss-Webster mice in the facilitation of this type of learning task. Previous work by Hunt and Krivanek (1966) had placed this range between 8 and 20 mg/kg of pentylenetetrazol injected i.p. for Wistar rats in a similar learning task. As in the previous two studies, animals were injected immediately i.p. after the last daily trial and the next set of trials was

begun 24 hours after injection. After the last daily trial, the mice received either saline or pentylenetetrazol solution. The doses of pentylenetetrazol were 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, and 20.0 mg/kg. It was found that variation in dose significantly affected the rate of learning. In addition, it was found that the means for the saline, 2.5 mg/kg, and 5.0 mg/kg groups were not significantly different. The rate of learning was found to be significantly higher for all other dose levels than for these three groups. Although 15 mg/kg appeared to be the most facilitative dose, its mean was not significantly different from the means of either the 10, 12.5, or 20 mg/kg dose conditions. Finally, learning was enhanced at all doses above 7.5 mg/kg.

(4) Again using an appetitively motivated visual discrimination task in a Y-maze, an attempt was made to determine the effect of time of drug administration on learning enhancement. The results of the three preceding experiments indicate that in mice, learning was enhanced by pentylenetetrazol administered immediately after each day's training trials. The mice were divided into ten groups. The control group received a daily i.p. injection of saline. The other groups received daily injections of 15 mg/kg pentylenetetrazol. As in study (3), this dose was found to be a highly facilitating dose. Different groups received the drug 60, 30, 15, and 5 minutes before

the first daily trial, or 1, 5, 15, 30, and 60 minutes after the last daily trial. The saline injections were given to the control group both before and after the trials, using the drug injection schedule described above in a random manner from day to day. An analysis of the results showed that the time of injection significantly affected learning. Learning was most facilitated in animals given injections shortly before or after training trials. The best group appeared to be the 5-minute posttrial group. The means of the 1-minute posttrial, 5-minute pretrial, and the 15-minute pre- and posttrial groups did not significantly differ from the mean of the 5-minute posttrial group. All these groups differed significantly from the saline group and the remainder of the time-interval groups. The means of the saline, 30-minute posttrial and 60-minute pre- and posttrial groups did not significantly differ from that of the 30-minute pretrial group. Therefore, maximal facilitation occurred if the injection was given with the 15-minute pretrial and 15-minute posttrial interval. One thing not found by (4), which might be expected according to McGaugh (1966), is a gradient effect. It should be expected that the degree of facilitation would decrease directly with increases in the interval between the training and the posttrial injections. As expected, no significant facilitation occurred with the 30-minute and 60-minute posttrial injections. However, there was no facilitation

gradient in the 1-, 5-, and 15-minute posttrial groups.

Hunt and Bauer (1969), using a Y-maze, trained Wistar rats in a black-white discrimination, and then injected them i.p. with 7.5, 10.0 or 15.0 mg/kg of pentylenetetrazol or saline. Injections were given immediately or 15 minutes after training. The animals were retested 24 hours later. Significant effects were obtained for both main effects and their interaction, showing that the facilitation of learning was a joint function of the amount of drug and the time at which it was given. On the average, the drug groups performed better than the controls, and although the drug groups did not differ from each other, the effect of a particular dose depended upon the time of injection. The most reliable evidence for facilitation was obtained for 7.5 mg/kg with injection immediately after training, and for 10 mg/kg with injection delayed. Animals receiving 15 mg/kg pentylenetetrazol showed only marginal evidence of facilitation regardless of time of injection. No differences were indicated between the control groups, regardless of time of injection, suggesting that injection per se had no effect on retention. In the case of the 7.5 and 10 mg/kg groups, however, the time of injection was a significant variable.

In a second experiment, Hunt and Bauer (1969) again using a Y-maze and Wistar rats, trained the animals in a position discrimination task extending over a period of

several days. Following each day's session, animals were injected with 10.0 mg/kg pentylenetetrazol or saline at intervals of 0, 5 or 10 minutes. It was found that, for immediate injection, there was no difference between the saline and drug group. However, under delayed conditions, the drug significantly improved performance when compared to the saline controls. The drug, 10-minute group showed significantly more rapid learning than the saline controls and also showed superior performance to the drug, 0-minute group. The drug, 5-minute group did not significantly differ from the two other drug groups but occupied a position mid-way between them. What in essence occurred was a gradient effect going in the opposite direction to that proposed by McGaugh (1966). This finding is in direct conflict with Krivanek and McGaugh (1968) as well. However, the previous finding that increased facilitation of learning occurs with increasing doses of pentylenetetrazol only up to a certain point was confirmed (Krivanek & McGaugh, 1968).

Thus, at the present time there seems to be some doubt as to the facilitory efficacy of pentylenetetrazol as a function of delayed injections after a training trial. Also in doubt is whether a gradient effect exists, and if so, in which direction and under what conditions. Also uncertain is the nature of the Drug X Time interaction. To be sure, many confounding factors are involved. Included among these are those mentioned previously: type of animal

used, strain of animal used, range and number of drug doses used, range and number of time intervals used, type of task used, difficulty of task used, definition of training situations, and criteria used for assessing the facilitation of learning (Krivanek & McGaugh, 1968; McGaugh & Dawson, 1971). Recent research has pointed to some other confounding variables as well. The degree of deprivation of the animal, the sex of the animal, the level of shock used in avoidance procedures (Krivanek, 1971) and the number of injections that a given animal receives during training sessions before given a test of retention (Bauer, 1972).

What follows is an attempt to clarify the above problems, in order to achieve a better understanding of the "time-dependent" processes involved in memory storage.

CHAPTER II

METHOD

Subjects

The subjects were 36 male Sprague-Dawley CFE rats from Carworth Laboratories, approximately 97 to 109 days old and weighing approximately 310-370 g at the beginning of the experiment. The animals were randomly assigned to groups corresponding to four dosage levels; neutral saline (0.9%), and 10.0, 12.5 or 15.0 mg/kg body weight of pentylenetetrazol. Within each dose level, the groups were divided randomly into groups which received intraperitoneal injections at the following times after completion of the last training trial: 0 minutes (immediately), 5 minutes or 15 minutes. Thus, there were twelve experimental conditions in all, each condition having three Ss randomly assigned to it. The pentylenetetrazol doses were dissolved in neutral saline and each rat received the appropriate solution or neutral saline by being injected with .01 cm^3/g body weight of rat.

The rats were housed in individual cages. They were placed on the following eight-day deprivation schedule prior to the start of the experiment: days one through three - 10 g per day; days four through eight - 5 g per day. During the course of the experiment, each animal was given 5 g of food immediately upon return to its home cage. This

schedule reduced and maintained the animals at 80-85% of their normal body weight.

Apparatus

The apparatus employed during this experiment was a symmetrical Y-maze, whose three arms were joined together such that the angle between any two arms was 120°. This arrangement produced a triangular area at the center of the maze common to and extending beyond each arm. Each arm measured 55.6 cm long, 15.0 cm wide, and 14.0 cm high. Each arm contained a white plexiglass shield, 3 mm thick, set 8.5 cm from the unjoined end of the arm, and a masonite guillotine door, 4 mm thick, set 17.7 cm from the joined end of each arm. Behind each plexiglass shield was a 15-Watt incandescent light bulb. Both the sides and the floor of the maze were constructed of wood and painted gray. The portion of the maze from the guillotine doors to the center and the triangular center portion were covered by clear plexiglass. The rest of the maze was covered with three masonite doors hinged immediately behind the guillotine doors. Clear plastic food cups were located at the rear of each end chamber, immediate to the plexiglass shield. Each end chamber was 28.7 cm in length and was defined by the distance between the plexiglass shield and the guillotine door.

Suspended approximately 105 cm above the triangular center portion of the maze was a 25-Watt incandescent

light bulb.

Procedure

After the rats had been randomly assigned to one of the twelve experimental conditions, they were further randomly assigned a running order irrespective of their experimental conditions. This consisted of assigning each subject to one of four groups of nine Ss each, which were run consecutively. Since the actual experiment lasted four days, the four groups with respect to running order were staggered over a 16-day period.

On the first day, each rat was randomly placed in an end chamber in one of the arms of the Y-maze. After 30 seconds, the guillotine door was raised allowing the rat to move out of the end chamber into the choice point area of the Y-maze. Upon reaching either one of the other end chambers, the rat was rewarded with two 45-mg Noyes Food Pellets. While the rat was in the act of consuming the food pellets, the guillotine door behind the animal was lowered, thus constituting the end of a single trial. Thirty seconds after the guillotine door was lowered, it was raised again and the rat began its second trial. Each animal was given a total of ten trials on the first day. None of the lights behind the plexiglass shield was turned on, and no discrimination training occurred.

On the second day, each rat was given nine brightness preference trials in the apparatus. Before each trial, the

light bulb at the end of one arm was turned on. The right-left position of the lighted arm was systematically varied from trial-to-trial. The rat was rewarded whether it chose the lighted or non-lighted arm. If the rat chose the lighted arm, it remained lighted throughout the 30-second intertrial interval. Subsequently, the rat's brightness preference was determined.

On the third day, each rat was trained to run to the alley of non-preferred brightness. During the training phase, reinforcement was given only for a correct response. The light positions at the end of the arms of the maze were systematically varied. Training was considered complete when each rat had achieved a criterion of nine out of ten correct responses with a minimum of 15 trials. After the last trial on the third day, the rats were treated in accordance with the conditions to which they were assigned to at the beginning of the study. Those rats in the 0-minute group were lifted from the apparatus immediately after completion of their last trial and injected with the appropriate solution. Rats in the other time conditions were lifted from the apparatus after completion of their last trial and placed in a cage next to the experimental apparatus. They were removed again from this cage and injected with the appropriate solution after the appropriate amount of time had passed.

On the fourth day, a retention test was given.

During the retention phase, the rats were run for 15 trials, using a non-correction procedure, with reinforcement given only for a correct response. With the exception of a fixed number of trials, the retention test was a repetition of the training procedure given 24 hours earlier. The number of errors made by the animals on the fourth day was used as the measure of retention of training given on the third day.

CHAPTER III

RESULTS

The mean number of errors made by Ss in each of the twelve experimental conditions as well as the mean number of errors made by Ss in each level of drug and level of time of administration condition is shown in Table I (see also appendix A).

Significant effects were found for both the drug dosage level ($F=6.66$, $df=3/24$, $p < .005$) and the injection time ($F=10.41$; $df=2/24$, $p < .001$). Although both main effects were found to be significant, the Drug X Time interaction was found to be significant only at the .05 level ($F=2.68$, $df=6/24$, $.05 > p > .01$) (see appendix B). The drug dosage level accounted for 21.5%, the injection time 22.4%, and their interaction 17.3% of the total variance.

The primary planned comparison of interest was to determine if the control or saline groups differed from the drug groups. The results of this comparison were found to be significant ($F=10.55$, $df=1/24$, $p < .005$), showing that on the average the drug did indeed have a facilitative effect on retention at the doses investigated. Other orthogonal planned comparisons showed that on the average, Ss in the 12.5 and 15.0 mg/kg groups showed greater retention than Ss in the 10.0 mg/kg group ($F=7.82$, $df=1/24$, $p < .01$). However, no difference in retention was found between the

TABLE I

Mean Number of Errors as a Function of Dose Level and Injection Interval (n=3)

	Saline	10.0 mg/kg	12.5 mg/kg	15.0 mg/kg	Time Means
0 min	5.33	2.67	1.00	1.67	2.67
5 min	5.67	5.33	4.33	1.33	4.17
15 min	5.00	6.00	5.00	5.00	5.25
Dose-Level Means	5.33	4.67	3.44	2.67	

12.5 and the 15.0 mg/kg groups (F=1.40, df=1/24, $p > .05$).

The Tukey HSD method, using the Studentized Range Statistic (q), for unplanned comparisons failed to show a difference in retention between the 10.0 and the 12.5 mg/kg groups (q=2.65, df=2/30, $p > .05$) or the 10.0 and 15.0 mg/kg groups (q=4.31, df=3/30, $.05 > p > .01$), although the latter comparison is nearly significant at the .01 level (see appendix C). Other comparisons also showed that those Ss who were injected immediately performed significantly better than Ss in which injection was delayed by 15 minutes (q=6.42, df=3/30, $p < .01$), although there were no significant differences between the 0-minute group and the 5-minute group (q=3.73, df=2/30, $.05 > p > .01$) and the 5-minute group and the 15-minute group (q=2.69, df=2/30, $p > .05$). When the three different control conditions were compared, no differences in retention were found (saline-0 minute vs. saline-5 minute, q=0.42, df=2/24, $p > .05$; saline-0 minute vs. saline-15 minute, q=0.41, df=2/24, $p > .05$; saline-5 minute vs. saline-15 minute, q=0.83, df=3/24, $p > .05$). This suggested that injection per se had no effect on retention.

Injections of pentylenetetrazol delayed by 15 minutes were found to have no effect on retention. The mean of the saline or control groups is identical to the mean of the means of the three 15-minute-drug groups. In fact, it appears that the performance of the 10.0 mg/kg-15-minute

group was poorer than the saline controls. This difference, however, is not significant when tested by the Scheffé procedure (F=0.52, evaluated against (6) (F.01, 6/24, $p > .05$).

Both the drug dosage level and the injection time were shown to exhibit significant linear trends for the range of values investigated (F=16.20, df=1/24, $p < .001$ and F=19.40, df=1/24, $p < .001$, respectively). The fact that the time variable exhibits a linear trend is important in that it provides evidence for a gradient effect in that the efficacy of pentylenetetrazol, averaged over the three different doses employed in the experiment, in facilitating retention decreases linearly or along a gradient as the interval between the end of training and injection increases (see appendix D).

To determine if the number of errors on the retention test was influenced by the number of trials to criterion during training on the previous day, an analysis of covariance was run. The results of this analysis showed that after adjusting for trials to criterion the drug dosage level effect remained significant at the .005 level and the injection time effect remained significant at the .001 level (F=6.24, df=3/23, $p < .005$ and F=9.51, df=2/23, $p < .001$). However, the Drug X Time interaction was no longer significant at the .05 level (F=2.33, df=6/23, $p > .05$). Thus, it was concluded that the significant main

effects were not associated with the number of trials to criterion (see appendix E).

CHAPTER IV

DISCUSSION

In general, the results of this experiment confirm the findings of previous experiments that pentylenetetrazol when administered in subconvulsant doses facilitates learning presumably by exerting some effect upon memory consolidation processes located within the central nervous system. That these processes are time-dependent was shown by the fact that the efficacy of a particular dose of pentylenetetrazol in facilitating learning depended upon the immediacy of injection after training had been completed.

The results presented here are, for the most part, consistent with those of Krivanek and McGaugh (1968). In both instances the doses of 10.0, 12.5, and 15.0 mg/kg pentylenetetrazol proved to be facilitative in promoting increased retention of a similar appetively motivated visual discrimination task in a Y-maze. Krivanek and McGaugh (1968) report that although the 15.0 mg/kg dose appeared to have the maximal facilitative effect on retention, it was not significantly different from either the 10.0 or 12.5 mg/kg doses. This is in agreement with the findings of this experiment. The data employed by Krivanek and McGaugh (1968) was obtained from 48 male and 48 female mice. It is interesting to point out that when the results of the female mice are separated from those of the male

mice, the error scores of the males show a gradual decrease over the 10.0 and 15.0 mg/kg range, similar to the results found here.

With respect to the time of injection after training variable, both Krivanek and McGaugh (1968) and this study show increased retention when pentylenetetrazol was administered immediately or nearly immediately or after a five-minute period as compared to the saline controls. However, no evidence was found here to indicate that a delay of 15 minutes would still facilitate retention as was found by Krivanek and McGaugh (1968).

The most significant aspect of this experiment was that it was the first to demonstrate the gradient effect hypothesized by McGaugh (1966). As the degree of facilitation of memory was found to decrease with increases in the interval between the termination of training and injection of pentylenetetrazol over the range of dosages investigated, this experiment may be taken as supportive evidence as to the existence of such an hypothesized gradient. The results of this experiment clearly indicate a gradual decrement over increasing time intervals which can be described as a linear relationship.

Since the scores of the Ss in the three drug conditions with injection delayed by 15 minutes were shown not to be significantly different from the scores of the saline controls, it is presumed that the drug loses its

effectiveness when it is injected at or beyond this period of time after training has been completed.

The study of Hunt and Bauer (1969) represents the first attempt to study the effect of different doses of pentylenetetrazol given at different intervals after training within a single experiment. In many respects, the experimental design presented here is closely related to the first experiment presented in their paper. Consistent with previous research and the results presented here, they found that, although on the average, the drug groups of 7.5, 10.0, and 15.0 mg/kg did not significantly differ from each other, the drug groups performed significantly better than the saline controls. Contrary to the results presented here, Hunt and Bauer (1969) found a significant Drug X Time interaction at the .01 level of significance and the most facilitating time of injection to be dependent upon the particular dose of pentylenetetrazol that was injected. Although their finding that 7.5 mg/kg pentylenetetrazol with immediate injection facilitated retention may be construed as consistent with the results presented here, their findings of maximal facilitation with injections of 10.0 mg/kg delayed by 15 minutes and only a marginal facilitation at 15.0 mg/kg doses irrespective of time of injection may not.

If one examines the mean scores of the different experimental conditions presented in Table 1, it appears

that the 15.0 mg/kg-5-minute group showed increased retention when compared to the 10.0 mg/kg-0-minute group - a situation analogous to the results found by Hunt and Bauer (1969). This difference, however, is not significant (q=1.67, df=3/24, $p > .05$).

The discrepancies between the results found by Hunt and Bauer (1969) and those presented here might be explained in terms of the training procedure utilized in the two experiments. Hunt and Bauer (1969) used a training procedure in which each S was run for 15 trials regardless of the number of errors it made. The procedure employed here required that the Ss demonstrate that they had learned the task by achieving a criterion of nine out of ten correct responses, since most previous work of this type had trained its Ss to some sort of learning criterion. The mean trials and errors to criterion found here were 61.47 and 26.44, respectively. Since the discrimination task employed by both experiments was extremely similar, there is considerable doubt as to how well this task was learned by S in the experiment of Hunt and Bauer (1969). This difference may be reflected in the range of the means of the retention scores for the different experimental conditions. This study found a range of 1.00 to 6.00 mean errors as compared to 2.90 to 7.00 mean errors found by Hunt and Bauer (1969).

In the second experiment of Hunt and Bauer (1969), the finding of a negative gradient effect or that for a

10.0 mg/kg dose of pentylenetetrazol facilitation of retention increased as the delay of injection after training increased over a period of 0 to 10 minutes, is also difficult to explain. In this experiment, Hunt and Bauer (1969) after determining Ss preferences for one of the goal boxes in a Y-maze, trained them to run to the other goal box, thus establishing a position discrimination. The two experiments cited above from Krivanek and McGaugh (1968), the first experiment cited by Hunt and Bauer (1969), and this experiment were concerned with training Ss in a visual discrimination task. The use of a position discrimination task as opposed to a visual discrimination task may have accounted for a gradient effect going in an opposite direction to that predicted by McGaugh (1966) and found here.

When one compares the results obtained here with the results obtained by other experiments, it becomes necessary to take into account the list of confounding variables mentioned previously in the introduction section. Of particular relevance are the following variables: type, strain, and sex of animal, range of pentylenetetrazol doses and time intervals, and type, definition, and difficulty of training task employed. As has been shown, the results of the experiment using one set of the above variables cannot readily be used to predict the outcome of a similar experiment using another set of these variables. As the outcome of the experiment by Hunt and Bauer (1969) shows,

these variables may be more subtle than originally thought.

At the present time there exists a clear need to standardize the above confounding variables as well as others in experiments of this type. Such an attempt should prove fruitful in clarifying further the role of pentylenetetrazol as it relates to the facilitation of learning through its effects upon the memory consolidation processes.

SUMMARY

The effects upon retention of a brightness discrimination by pentylenetetrazol administered at different doses and at different time intervals after training was studied. Thirty-six rats were trained to run for food reinforcement in a Y-maze until they reached a criterion of nine out of ten correct responses. They were injected with either 10.0, 12.5 or 15.0 mg/kg pentylenetetrazol at intervals of either 0, 5, or 15 minutes after completion of training. Twenty-four hours later the rats were run for 15 more trials in the Y-maze under identical conditions. The number of errors made during these additional trials served as a measure of retention of what the rats had learned on the previous day.

The results indicated that the number of errors made was dependent upon the dosage of drug and the time intervals after training at which it was administered.

The most significant finding of this experiment was that of a gradient effect, which indicated that the effectiveness of pentylenetetrazol in facilitating retention decreased linearly with increasing delay of injection.

The results found here support previous research which also showed an over-all ability of pentylenetetrazol to facilitate retention of a learned task.

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APPENDIXES A, B, C, D, AND E

APPENDIX A

Number of trials to criterion by Ss during training arranged by dose level and injection interval

	Saline	10.0 mg/kg	12.5 mg/kg	15.0 mg/kg	Total
	100 (L)	62 (L)	72 (L)	55 (D)	
0 min	19 (D)	68 (L)	56 (D)	38 (L)	763
	72 (L)	97 (L)	105 (L)	19 (D)	
	102 (L)	67 (D)	15 (D)	20 (D)	
5 min	16 (D)	67 (L)	90 (L)	85 (L)	868
	155 (L)	47 (L)	84 (L)	120 (L)	
	78 (L)	21 (D)	61 (D)	45 (L)	
15 min	34 (D)	31 (D)	46 (L)	72 (D)	582
	107 (L)	42 (L)	19 (D)	26 (D)	
Total	683	502	548	480	

Note.--The designations (D) and (L) denote the arm of the Y-maze, dark or light respectively, to which each S was trained to run. Thus, these designations constitute the opposite of each S's brightness preference.

Number of errors made by Ss during retention testing as a function of dose level and injection interval

	Saline	10.0 mg/kg	12.5 mg/kg	15.0 mg/kg	Total
	5 (b)	4 (b)	0 (a)	2 (a)	
0 min	6 (b)	1 (b)	3 (b)	1 (a)	32
	5 (c)	3 (c)	0 (d)	2 (c)	
	6 (c)	4 (c)	3 (a)	2 (a)	
5 min	5 (d)	5 (d)	4 (c)	2 (b)	50
	6 (d)	7 (d)	6 (d)	0 (d)	
	7 (a)	5 (b)	5 (a)	6 (a)	
15 min	5 (a)	5 (b)	4 (b)	6 (c)	63
	3 (d)	8 (d)	6 (c)	3 (c)	
Total	48	42	31	24	

Note.--The designations (a), (b), (c), and (d) represent from which group with respect to running order each score was obtained.

APPENDIX B

ANALYSIS OF VARIANCE - TWO-FACTOR COMPLETELY RANDOMIZED DESIGN

Source	df	MS	F
Total	35		
Drug level	3	12.92	6.66**
Time	2	20.20	10.41***
Drug X Time	6	5.19	2.68*
Error (within cells)	24	1.94	

*$p < .05$

**$p < .005$

***$p < .001$

APPENDIX C

Summary of Specific Comparisons

Comparison			F or q
Saline-pooled	vs.	10.0, 12.5, 15.0-pooled	10.55***
12.5, 15.0-pooled	vs.	10.0-pooled	7.82**
12.5-pooled	vs.	15.0-pooled	1.40
10.0-pooled	vs.	12.5-pooled	2.65
10.0-pooled	vs.	15.0-pooled	4.31*
0 min-pooled	vs.	15 min-pooled	6.42**
0 min-pooled	vs.	5 min-pooled	3.73*
5 min-pooled	vs.	15 min-pooled	2.69
Saline-0 min	vs.	Saline-5 min	0.42
Saline-0 min	vs.	Saline-15 min	0.41
Saline-5 min	vs.	Saline-15 min	0.83
10.0, 12.5, 15.0-15 min	vs.	Saline-pooled	0.00
10.0-15 min	vs.	Saline-pooled	0.52
15.0-5 min	vs.	10.0-0 min	1.67

*$p < .05$

**$p < .01$

***$p < .005$

APPENDIX D

TREND ANALYSIS -

SUMMARY TABLE FOR MAIN EFFECTS

Source	df	MS	F
Drug level	3		
Drug level (linear)	1	31.43	16.20*
Drug level (quadratic)	1	6.46	3.32
Drug level (cubic)	1	0.86	0.44
Time	2		
Time (linear)	1	37.13	19.14*
Time (quadratic)	1	3.16	1.63
Error (within cells)	24	1.94	

*$p < .001$

APPENDIX E

ANALYSIS OF COVARIANCE - SUMMARY TABLE

Source	df	SS	MS	F
Total	34	154.91		
Drug level	3	37.80	12.60	6.24*
Time	2	38.42	19.21	9.51**
Drug X Time	6	28.25	4.71	2.33
Error (within cells)	23	46.57	2.02	

*$p < .005$

**$p < .001$